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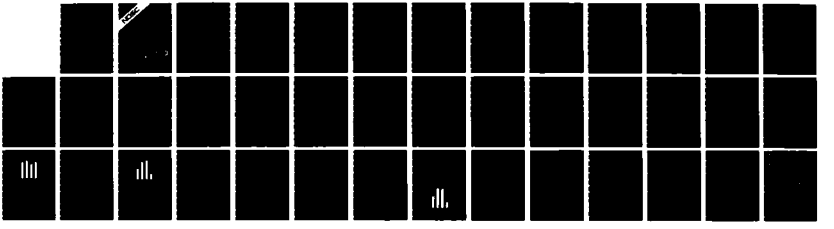

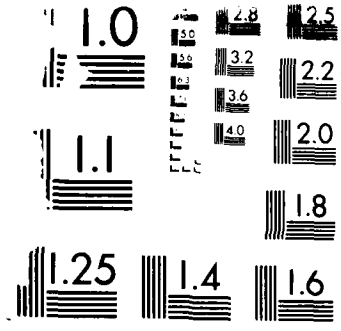
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Acute and Chronic Effects of Tributyltin on the Mysid *Acanthomysis sculpta* (Crustacea, Mysidacea)

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Acute and Chronic Effects of Tributyltin on the Mysid Acanthomysis sculpta (Crustacea, Mysidacea)

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Acute and chronic toxicity testing with tributyltin (TBT) leachate derived from panels painted with antifouling paint was performed using the mysid species Acanthomysis sculpta (Crustacea, Mysidacea). Tests included a 96-hour acute static renewal, a long-term (63-day) mortality test, a series of growth tests in which length and freeze-dried weight were measured, and a reproductive success test. All tests except the 96-hour acute study were performed in a flowthrough dosing regime ranging in concentrations from 0.03- to 0.52-μg/L TBT. The acute test was performed within a dose range of 0.25- to 0.66-μg/L TBT. In all experiments, the dose levels were measured by hydride derivatization and atomic absorption detection.

A 96-hour LC_{50} value for juveniles was determined at 0.42-μg/L TBT. Reproductive effects were the most sensitive sublethal indicator of TBT toxicity. A chronic value of 0.14-μg/L TBT was determined above which a significantly reduced release of viable juveniles was apparent. Length and weight, in adult females, were affected in concentrations above 0.31-μg/L TBT. At higher concentrations the rate of mortality throughout a life cycle was significantly greater than that of controls. These results suggest that concentrations of TBT >0.14-μg/L affect the ability of the mature female to grow and reproduce. Based on the 96-hour LC_{50} and the most sensitive chronic value measured, reproduction, an acute chronic ratio of 2.9 was calculated. (Continued)

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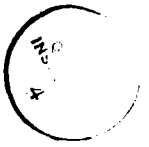
A comparison of TBT toxicity was performed using a TBT solution leached from painted panels and one derived from a pure TBT compound. An acute dose of each was used and mortality curves throughout the 168-hour test proved similar, suggesting no synergistic effects from the copper toxicant in the coating.



EXECUTIVE SUMMARY

Acute and chronic toxicity tests using organotin antifouling (AF) coating leachates were performed with the mysid *Acanthomysis sculpta*. Tests included a 96-hour acute static renewal, a long-term (63-day) mortality test, a series of growth tests, and a reproductive success test. All tests, except the 96-hour acute, were performed using a flowthrough tributyltin (TBT) dosing system ranging in concentrations from 0.03- to 0.52-µg/L TBT. The acute test was performed within a dose range of 0.25- to 0.66-µg/L TBT.

ACUTE TOXICITY TEST

The LC_{50} was determined at 0.42-µg/L TBT for juvenile *A. sculpta*. An LC_{50} value of 0.61-µg/L TBT had previously been estimated for juvenile *A. sculpta* using a flowthrough dosing system. Observations made during a 72-hour recovery phase following the 96-hour test period showed mortality ceased or slowed dramatically at doses below the newly established LC_{50} of 0.42-µg/L TBT, while individuals exposed in doses above the LC_{50} continued to die. At the end of the 72-hour recovery phase, all organisms had died at the 0.66- and 0.54-µg/L TBT dose levels and only 4 percent survived at 0.43-µg/L TBT.

In conjunction with the acute toxicity test, a spike test and a test comparing the effects of a TBT leachate solution and a pure TBT compound solution were performed. The spike test examined the effects on mysids of an 8-hour elevated exposure to 1.13-µg/L TBT after exposure in a subacute concentration of 0.25-µg/L TBT for 44 hours. After the 8-hour spike, the concentration was returned to the subacute level. Mysids exposed to the acute TBT spike showed a marked increase in mortality during the spike and recovered at a slower rate than those not exposed to the spike. At the end of the recovery phase, 52 percent survived the subacute exposure with the spiked dose, while 76 percent survived the subacute exposure only. All surviving individuals, however, appeared to be healthy and to have recovered successfully.

The pure compound/leachate comparison examined mysids held at an acute concentration (0.48-µg/L TBT) in TBT solutions established with both leachate stock solution and a stock solution mixed from a pure TBT compound. Mysids held within these two test conditions exhibited similar mortality curves and failed to recover after the TBT dosing ceased. At the end of the test, 4 percent of the mysids dosed with the leachate survived compared to 16 percent dosed with the pure compound. These results suggest no synergistic effects from the copper in the AF leachates occurred.

LONG-TERM MORTALITY TEST

The long-term mortality test was performed to monitor the survival of mysids exposed to TBT throughout a life cycle. This test was initiated with juvenile mysids less than 24 hours old. TBT test concentrations were maintained below 0.61-µg/L TBT, the previously established and best estimated 96-hour LC_{50} value for juvenile mysids. Within the first 22 days and before the mysids reached sexual maturity, all individuals in the highest dose level

(0.48 µg/L) had died. After 36 days, when sexual maturity had been reached, individuals in the next lowest concentration (0.38 µg/L) showed an increase in mortality over mysids exposed to lower TBT concentrations. Only 22.5 percent of the individuals in 0.38 µg/L survived to day 63 compared to 60 percent survival in the controls. The subsequent static renewal 96-hour LC_{50} estimate of 0.41-µg/L TBT indicated that the previous 96-hour LC_{50} estimate of 0.61-µg/L TBT was likely too high. The degree of mortality noted in the long-term test at 0.48-µg/L TBT supports this observation.

GROWTH

Growth measurements of length and freeze-dried weight were performed with surviving individuals in the mortality test. In addition, three separate growth tests were performed in which similar length and weight measurements were made periodically throughout a life cycle. Dose levels were compared by day, and because adult male and female mysids differ in size considerably, the sexes were analyzed separately after sexual maturity was reached.

The most significant effect observed in the growth tests was seen in sexually mature females at the higher test concentrations. The 63-day-old female survivors from the mortality test in the 0.48-µg/L concentration showed a statistically significant difference in both length and weight from the rest of the test animals in all concentrations. Length and weight differences were seen in 28-day-old females at the 0.49-µg/L TBT level in growth tests. In each instance, no clear significant differences could be seen in the corresponding males.

REPRODUCTIVE LIFE CYCLE TEST

A test investigating the reproductive sensitivity of this mysid to TBT was conducted over a 53-day period approximating one life cycle. The number of juveniles released from 14 select females raised in each of four concentrations and a seawater control were counted, and the amount of time from birth of the female to release of its juveniles was noted. Also, the number of individuals in each unhatched brood was enumerated from the remaining 14 select females, as well as from a stock of females hatched at the same time and raised together with the select females under identical test conditions.

No significant statistical differences were found in the number of juveniles released per female, the number of individuals in unhatched broods, or the number of days from the hatching of a female to the release of its juveniles. However, there was a marked difference in the number of females that released juveniles. Of the select females held individually in each concentration, none released juveniles at 0.33 µg/L, 23 percent released at 0.19 µg/L, 85 percent released at 0.09 µg/L, 71 percent released at 0.03 µg/L, and 46 percent released in the controls.

A final chronic value would lie between the lowest dose level where an effect was observed and the highest dose level where no effect was observed. For long-term mortality that value would be between 0.25 and 0.3 µg/L. For

growth, it would be between 0.25 and 0.38 µg/L. And for reproduction, the final chronic value would lie between 0.09- and 0.19-µg/L TBT.

Using the acute value and chronic ranges determined, an acute/chronic ratio of 3.0 was calculated for the mysid *A. sculpta*.

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INTRODUCTION

Tributyltin (TBT) is commonly used as an antifouling toxicant in marine paints and, hence, may enter the marine and freshwater environment directly by leaching processes. Recent reports have documented widespread presence in aquatic systems (Maguire et al., 1982; Thompson et al., 1985; Mueller, 1984; Valkirs et al., 1986). Additionally, toxicity to a wide range of organisms has also been reported, in some instances at concentrations below 1-µg/L (Ward et al., 1981; Waldock & Thain, 1983; Laughlin et al., 1984; Salazar & Salazar, 1985; U'ren, 1983; Beaumont & Budd, 1984; Walsh et al., 1985; Valkirs et al., 1985a).

Recently, national attention has been focused on the potential effects that TBT leachates may have on the marine environment both in Congress (Senate Resolution 272, Congressional Record - Senate, S17543, 12 Dec 85) and the US Environmental Protection Agency (EPA) (Special Review. Federal Register, Vol 51, No. 5, p 778, 8 Jan 86). Proposed use of TBT-containing coatings by the Navy has further stimulated interest in the potential effects on nontarget organisms. In response to the National Environmental Policy Act and the need to develop a risk assessment, the studies reported herein provide long-term toxicity data on a sensitive planktonic crustacean.

Improved testing strategies supported by improvements in analytical chemistry have permitted better short-term and long-term estimates of TBT toxicity to nontarget organisms. Such studies have demonstrated that TBT is a slow acting toxicant in some instances and that short-term tests may result in unrealistic high toxicity values, which tend to underestimate TBT toxicity by setting artificially high levels (Laughlin et al., 1982). Clearly, studies that provide long-term toxicity data are essential to assess ecological threats to nontarget organisms posed by TBT, and are necessary for the development of water quality criteria and standards. No Observable Effects Levels (NOEL), which define the highest concentration where no effect was observed and the lowest concentration where significant effects were observed, may be estimated from such studies.

When long-term testing is possible with sensitive species, meaningful toxicity estimates may be generated that accurately predict potential threats to survival. The short-term and long-term toxicity, sublethal growth, and reproductive tests summarized in this report discuss toxic effects of TBT to the marine mysid shrimp, *Acanthomysis sculpta* (Crustacea, Mysidacea), a sensitive marine species suitable for long-term growth and survival testing.

METHODS

TEST ANIMAL

The mysid *Acanthomysis sculpta* (Crustacea, Mysidacea) was used as the test animal in these assessments for a variety of reasons. It has proven to be a sensitive and reliable species in over 30 bioassays conducted by the Naval Ocean Systems Center (NOSC) to qualify dredge material from Navy projects in San Diego Bay for ocean disposal. Mysids are sensitive to a

variety of toxicants (Salazar & Salazar, 1985) and reliable in terms of experimental repeatability (EPA/ACE, 1977). Its importance in the marine food chain as forage for fish (Hobson & Chess, 1976) and its availability and capacity for culture in the laboratory also influenced selection of this test species.

Distribution of the genus *Acanthomysis* is known to be worldwide; however, *A. sculpta* has only been found in the eastern Pacific close to the American coast (Ii, 1964; Tatersall, 1932, 1951; Banner, 1948, 1954; Mauchline, 1977). This species generally schools in close association with the bottom sediment during the day and migrates toward the surface at night (Hobson & Chess, 1976). Also, *A. sculpta* is frequently associated with the kelp canopy of *Macrocystis pyrifera* and remains at the surface, active and feeding, during the day and night. They appear to be sight feeders, feeding more actively during the day (Green, 1970).

A reproductive cycle of *A. sculpta* can range anywhere in duration from 30 to 60 days depending on environmental conditions, but females usually release free-swimming juveniles within 40 days. Clutter and Theilacker (1971) report a life cycle duration of 63 days for the mysid *Metamysidopsis elongata*, and Nimmo et al. (1978) found that *Mysidopsis bahia* released young within 19 days of birth. Eggs are fertilized and develop within a marsupium, or brood pouch, consisting of lamellae present on the thoracic legs. The eggs hatch within the brood pouch and develop through four larval stages before being released as fully developed juveniles. Green (1970) defines the stages as: Ia (24 hours after hatching), Ib (48 hours after hatch), Ic (60 hours after hatch), II (86 hours after hatch), and III (134 hours after hatch). Five to six days after eggs are extruded into the brood pouch and fertilized, they hatch, and after another five to six days of larval development, they are released from the brood pouch as actively swimming juveniles proceeding through several molts during development to mature adulthood.

Brood sizes vary considerably. Green (1970) noted from 5 to 46 individuals in the brood pouches of *A. sculpta* collected within 1 meter of the bottom off Vancouver Island, British Columbia. We have observed an average brood size of 13.6 with a range of 3 to 26 for specimens collected from the surface kelp canopy off San Diego.

For tests summarized in this report, we collected *A. sculpta* from within the *Macrocystis pyrifera* kelp canopy off the western tip of Point Loma, San Diego, in the vicinity of the test facilities seawater intake. While in a small boat, we made collections with buckets from the surface water and transferred mysids to the laboratory holding system. We held these field-collected mysids under continuous flowthrough conditions with aeration. Within 12 hours, gravid females were sorted and placed in flowthrough rearing tanks that collected and isolated newly released juveniles.

Juveniles are released primarily at night. Green (1970) suggested because *A. sculpta* appear to be sight feeders and will eat their young, nocturnal liberation of the young is of considerable survival value. We found this behavior conducive to laboratory tests. Juvenile collection chambers were cleaned in the evening and checked the following morning for newly released juveniles. In this way, all mysids released over a 12-hour period

were collected representing essentially the same age class. We held a large number of gravid females in the rearing tanks to assure a sufficient number of juveniles for testing.

TEST FACILITIES

The testing facilities used for TBT toxicity testing with the mysid *A. sculpta* were described by Valkirs et al. (1985a). Briefly, they consist of a mobile bioassay laboratory equipped with a flowthrough seawater system. The TBT toxicant dosing system is capable of delivering five separate dose levels and a seawater control under continuous flowthrough conditions. The seawater intake for the lab is approximately 250 meters offshore from the NOSC/Marine Sciences Laboratory, in the same vicinity where test organisms were collected.

Tributyltin is introduced into the system as a leachate from panels coated with antifouling paint (International Paint Corp. SPC 954) containing a tributyltin polymer and cuprous oxide toxicants. Dose levels are determined by the amount of panel surface area exposed to fresh, incoming seawater. The rate at which TBT leaches from painted surfaces is known to be relatively constant and is related to water temperature, age, and preconditioning of the surface paint (Lieberman et al., 1985).

Because of water temperature variation, aging of the panels and the differential accumulation of algal and bacterial slime layers within the dosing systems and paint panels during the course of these tests, the amount of painted surface area was varied in some dose levels to maintain appropriate test concentrations. The concentration of TBT in all dose levels was measured once or twice per week (Valkirs et al., 1985b), and each dose level was treated separately in determining the amount of painted surface area required to achieve the desired concentration. Flow rates of seawater running over the painted surfaces were varied slightly to compensate for seasonal temperature variations to maintain the desired TBT concentration.

During the first tests conducted this year, the amount of painted surface area in each dose level was 177, 446, 1,115, 2,787, and 1,858 square centimeters corresponding to averaged dose concentrations of 0.03-, 0.08-, 0.24-, 0.39-, and 0.43-µg/L TBT, respectively. During the later tests, the three highest dose levels were reconfigured due to a drop in dose concentration. After adjustment, the amount of painted surface area was 177, 446, 929, 1,115, and 2,787 square centimeters corresponding to average dose concentrations of 0.04-, 0.10-, 0.21-, 0.33-, and 0.58-µg/L TBT, respectively. Actual average dose concentrations for all tests are presented in table 1.

Table 1. Summary of average TBT concentrations in µg/L as tributyltin chloride.

Dose	n*	Mean	Standard deviation
96-hour static renewal acute toxicity test			
1	8	0.25	0.08
2	8	0.31	0.12
3	8	0.43	0.16
4	8	0.54	0.15
5	8	0.66	0.17
Spike	8	0.25	0.09
Pure compound	8	0.48	0.21
Long-term mortality test and growth test no. 1			
1	14	0.03	0.01
2	14	0.08	0.02
3	17	0.25	0.17
4	16	0.38	0.13
5	15	0.48	0.18
Mysid growth test no. 2			
1	4	0.03	0.01
2	4	0.08	0.03
3	4	0.15	0.04
4	4	0.27	0.07
5	4	0.49	0.14
Mysid growth test no. 3			
1	6	0.05	0.02
2	6	0.13	0.05
3	5	0.21	0.11
4	6	0.33	0.11
5	6	0.59	0.34
Mysid reproduction test			
1	5	0.03	0.01
2	6	0.09	0.03
3	5	0.19	0.04
4	5	0.33	0.18
5	5	0.52	0.18

* n = number of TBT values

TEST PROCEDURES

The *A. sculpta* chronic bioassays consisted of a progressive series of tests designed to elucidate the area of TBT-related stress on the mysid as these areas became better defined. Long-term lethality was examined first followed by the effects of TBT on growth and, finally, reproductive ability. All tests were initiated with juveniles released from gravid females within 48 hours. Gravid females were collected fresh from the field prior to each test.

All test organisms were fed brine shrimp nauplii, *Artemia salina*, daily at or above maintenance levels (30+ nauplii/mysid). Flowthrough test containers were designed with a fine mesh screen (202-micron Nitex) covering the drain ports, which retained the brine shrimp nauplii as well as the test organisms. This resulted in the food source being constantly available to the test organisms.

Acute Toxicity Test

A 96-hour static renewal acute toxicity test was performed using *A. sculpta* juveniles less than 24 hours old. A nominal dosing regime consisting of five dose levels ranging from 0.3- to 0.8-µg/L TBT was established by diluting a TBT leachate of greater concentration than 0.8 g/L obtained from the bioassay laboratory flowthrough dosing system. Fresh dose water was mixed and introduced every 24 hours, and live/dead counts were made at that time. Chemical TBT measurements were made of each initial test solution in each dose and at the end of the 24-hour-exposure period by borohydide derivatization and atomic adsorption detection (Valkirs et al., 1985b). After 96 hours, all individuals were placed in clean, undosed, flowthrough seawater and observed for an additional 72 hours.

Five replicates of five individuals each were held in 250-ml containers in each dose level. Containers were filled with 200-mls test solution and were siphoned down to 25 mls every 24 hours when the solution was renewed. Siphoning was performed with a 1/4-inch Teflon tube fixed with a 202-micron Nitex intake screen. The screen prevented test organisms and food from being removed from the container. Any debris adhering to the screen was removed from the container. The siphoning flow rate was set low enough to allow the test mysids to avoid the screen by swimming against the induced current.

In conjunction with the acute toxicity test, a spike test and a test comparing the effects of a TBT leachate solution and a pure TBT compound solution were performed. The spike test examined mysids held at a concentration of 0.25-µg/L TBT that were exposed to an elevated dose, or spike, of 1.13-µg/L TBT for 8 hours and then returned to the lower dose. Five replicates of five individuals each were tested simultaneously with the five replicates of the lowest dose of the acute toxicity test, which acted as the control for the 8-hour spike test.

The pure-compound/leachate comparison examined mysids held near 0.43-µg/L TBT established with both leachate stock solution and a stock solution mixed from a pure TBT compound. Five replicates of five individuals each were held in solutions of pure TBT compound simultaneously with five replicates from the similar leachate derived dose level of the acute toxicity test. All repli-

cates were treated similarly; however, one set was held in a leachate solution and the other in a solution of only TBT.

Long-Term Mortality

The long-term mortality test was conducted for 63 days, a period greater than the reproductive cycle of *A. sculpta*. Five dose levels and a seawater control were used. Ten newly released juveniles were placed in each of five replicate containers resulting in a total of 50 mysids per dose level. Replicate polycarbonate test containers held 500 ml of water. Flow rates into each container were approximately 200 ml/min. Ten additional replicate containers of 10 individuals each were held in control conditions to better estimate natural mortality in the laboratory environment.

Concentrations of TBT in the five dose levels averaged 0.03, 0.08, 0.25, 0.38, and 0.48 µg/L during the 63-day test (table 1).

Mortality counts were taken infrequently during the course of the test to minimize disturbance and agitation of the test organisms. Data for analyses were taken from day 22, day 41, and day 63 because of the significance of each of these days in the course of the test. Day 22 was the first day the mysids were large enough to count actual live individuals without disturbing them significantly. Counts prior to this day were of dead individuals and only when observed. Day 41 was the last day survival counts were made prior to the release of juveniles by gravid females. Mortality in females could increase due to juvenile release. Day 63 was the last day survival observations were made prior to termination of the test.

Upon termination, all surviving mysids were measured for length and weight. Individuals were anesthetized with MS-222 (tricaine methanesulfonate) to stop movement but avoid shrinkage and distortion from immediate preservation. Individuals were measured under a Bausch and Lomb dissecting scope using an ocular micrometer.

Individuals were then rinsed with distilled water, blotted, and placed in separate, micro centrifuge tubes modified to allow air to pass through the cap. Tubes were labeled for individual identification. They were then placed in a freeze-dryer for at least 24 hours. Upon removal from the freeze-dryer, tubes were placed in a desiccator, and individuals were removed one at a time and weighed on a Cahn gram electrobalance within 8 hours. Care was taken to handle all specimens similarly during the weighing procedures to minimize any variations in weight due to hydration.

Growth Test I

Concurrent with the long-term mortality test, a separate growth test was performed. Eighty mysids were placed in 10-liter polycarbonate aquaria. One aquarium was held in each dose level. Initial length measurements were made of 30 individuals, and, periodically, 8 to 10 individuals were randomly sampled from each dose level to be sacrificed and measured. Length measurements were taken on days 6, 14, 27, 39, and 48. Individuals were also sexed on day 39 and 48, after they reached sexual maturity. Average TBT concentra-

tions for this test were the same as those determined in the 63-day mortality test (0.03-, 0.08-, 0.25-, 0.38-, and 0.48-µg/L TBT).

Growth Test II

A second growth test was conducted using 7 replicates per dose level with 10 to 15 newly released individuals per replicate. Replicates were held in polycarbonate test containers containing 500 ml of water. Flow rates into each container were approximately 200 ml/min.

At the beginning of the test, 31 juveniles were measured for length and discarded. At each sampling interval, one replicate from each dose level was sampled, and all individuals within the replicate were measured for length and weight as in the previous test. This method of sampling entire replicates avoided any error associated with randomly sampling a portion of the individuals from a common container, as was done in the previous test.

Mysids were sampled on days 10 and 28 of the test. On day 28, in addition to length and weight measurements, individuals were also sexed. TBT concentrations during this test averaged 0.03, 0.08, 0.15, 0.27, and 0.49 µg/L.

Combined Growth Test III and Reproduction Test

A test was performed that measured growth until individuals became sexually mature then focused on reproductive success. Five replicates were placed in each dose level with each replicate containing fifteen 12- to 24-hour-old juveniles. An initial sampling of 23 juveniles was taken for length and weight measurements. During days 8 and 21 of the test, one replicate from each dose was sampled, and individuals were measured for length and weight as in the previous tests. Upon reaching sexual maturity, gravid females were separated and placed individually into test containers holding 500 ml of seawater. Their condition was monitored daily until they released juveniles. At this time the newly released juveniles were counted. The percent of these females that released viable juveniles and the number of juveniles released per female were indices used to measure reproductive success.

TBT concentrations during this test averaged 0.05, 0.13, 0.21, 0.33, and 0.59 µg/L, and the test ran for 51 days.

Reproduction Test

The reproduction portion of the previous test was repeated, observing greater numbers of gravid females. Approximately 200 newly released juveniles were placed in each dose level in five separate 500-ml polycarbonate replicate containers per dose. They were maintained as in previous tests until they reached sexual maturity, at which time 14 gravid females were removed from each dose level and each was placed in a separate 250-ml polycarbonate test container. Their condition was monitored daily until juveniles were released, at which time newly released juveniles were counted. After a female released juveniles and the juveniles were counted, the female was placed back into its individual test container and observed daily until the end of the test.

The test was terminated when all of the 14 females held in each dose level had either released juveniles, aborted its brood, or showed no further signs of brood development. Upon termination, all test females were examined under a Bausch and Lomb dissecting scope and measured for length using an ocular micrometer.

In addition to observations of test females, all remaining gravid females in the dosed stock groups were examined. A brood count was made of each female, the stage of brood development was noted, and specimen lengths were measured.

The indices examined in determining reproductive stress due to TBT were (1) the number of females that released viable juveniles, (2) the number of juveniles released per female, (3) the number of days from hatching out of the female to the females' release of juveniles, a reproductive cycle, and (4) the brood count of females that had not released juveniles. The stage of the broods not yet released was also noted, as well as the condition of all females.

TBT concentrations during this test averaged 0.03, 0.09, 0.19, 0.33, and 0.52 µg/L, and the test ran for 54 days.

RESULTS

ACUTE TOXICITY

Toxicity at the dose levels tested proved to be directly dose dependent for a 96-hour period. Percent survival throughout the test is presented in table 2. At 96 hours, there was 92 percent survival in the controls, 76 percent at 0.25-µg/L TBT, 68 percent at 0.31-µg/L, 48 percent at 0.43-µg/L, 32 percent at 0.54-µg/L, and 20 percent at 0.66-µg/L (figure 1). From these data, an LC_{50} value of 0.42-µg/L TBT was derived by means of a probit analysis (SAS User's Guide: Statistics, 1982). The lower and upper 95-percent fiducial limits about this value were 0.36- and 0.49-µg/L TBT, respectively.

Table 2. Percent survival throughout 96-hour static renewal acute toxicity test.

	Dose (µg/L TBT)					
Hour	Control	0.25	0.31	0.43	0.54	0.66
0	100	100	100	100	100	100
24	100	92	96	100	100	100
48	100	92	96	92	96	100
72	96	88	84	72	72	88
96	92	76	68	48	32	20
120	92	76	68	36	12	0
144	92	76	64	8	0	0
168	92	76	60	4	0	0

Figure 1 indicates that survival was not clearly dose dependent prior to 96 hours. Individuals in the highest dose level, 0.66-µg/L TBT, showed no

mortality until 72 hours and exhibited a sharp drop in survival immediately after that. In contrast, the 0.54- and 0.43-µg/L TBT dose levels exhibited a sharp drop in survival 24 hours prior to the similar response in the highest dose level. This is a result of presenting only mortality data. The actual condition of individuals followed a more dose-dependent response throughout the test.

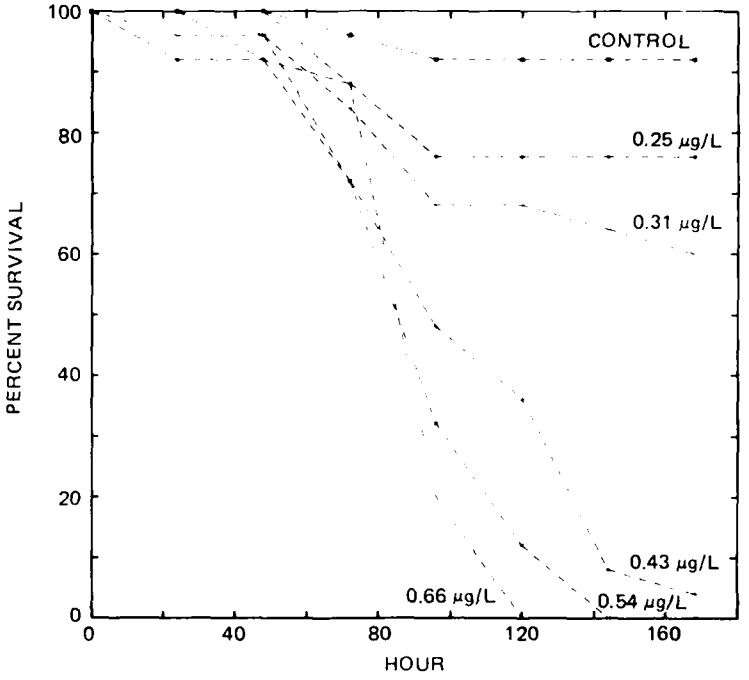

Figure 1. Percent survival through 96-hour static renewal acute toxicity test.

Previous tests with *A. sculpta* showed that acute TBT doses usually took 1 to 5 days to exhibit a mortality response. During that time, individuals exhibited a stressed condition through slow movement, aberrant swimming behavior, and, in many cases, no movement unless prodded. Such a response was observed in this test at dose levels of 0.43-, 0.54-, and 0.66-µg/L TBT.

Observations made after the 96-hour acute test, when surviving test specimens were placed in clean flowthrough seawater, showed mysids in dose levels below the 0.42-µg/L TBT LC_{50} were able to recover from any toxic effects while specimens in dose levels above 0.42 µg/L did not recover and died.

Exposure time directly affected this specie's ability to recover, as shown in the short-term spike test run in conjunction with the acute toxicity test. The group of mysids held at a subacute dose level of 0.25-µg/L TBT for 44 hours, then exposed to an 8-hour acute dose of 1.13-µg/L TBT, and returned again to the lower dose level had a higher initial mortality rate during the spike than mysids in the control group held at the lower level but not spiked (table 3, figure 2). However, the test group recovered to a similar mortality rate noted in the control group at the completion of the 96-hour dosing

period. Mortality in the test group and the control stabilized after TBT dosing ceased and clean, flowthrough seawater was introduced. At the end of the 168-hour observation period, mortality had ceased, and the condition of mysids in both the test and control group appeared normal.

Table 3. Percent survival throughout spike test. Control remained constant at 0.25-µg/L TBT while spike was held at 0.25 µg/L and elevated for 8 hours to 1.13-µg/L TBT.

	Dose	
Hour	Control (0.25-µg/L TBT) constant	Spike (1.13-µg/L TBT) 8-hour dose
0	100	100
24	92	96
44		96
48	92	
52		92
72	88	72
96	76	64
120	76	64
144	76	52
168	76	52

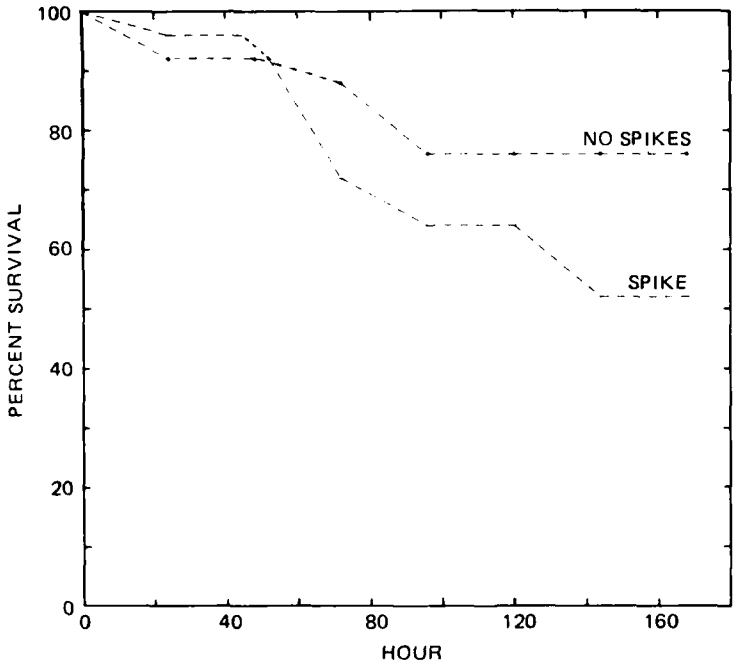

Figure 2. Percent survival through 96-hour static renewal acute toxicity test, 8-hour spike of 1.13 ppb at 44 hours.

Results from the leachate/pure compound comparison are illustrated in figure 3 (table 4) and show mortality to be essentially similar throughout the dosing phase of the test. Additionally, mysids did not recover from either the leachate or pure compound solution after dosing ceased and clean seawater was introduced. The dose level of 0.43-µg/L TBT was close to the concentration (0.42 µg/L) from which these mysids did not recover after a 96-hour exposure determined in the acute toxicity test. At the end of the 168-hour observation period, only 16 percent survival was recorded in the pure compound solution and only 4 percent survival was noted in the corresponding leachate solution. An analysis of covariance was performed using time as the covariate and testing the number dead between treatments. Even though survival was slightly higher in the pure compound solution, no statistically significant difference was found (a=0.05) between mortality in either solution throughout the 168 hours of observation.

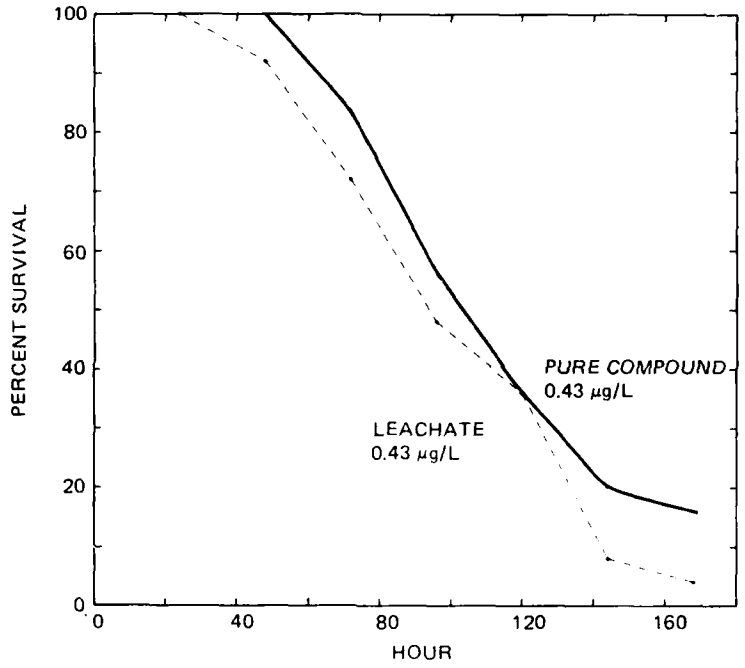

Figure 3. Percent survival through 96-hour static renewal acute toxicity test, pure compound versus leachate.

Table 4. Percent survival throughout leachate/ pure compound comparison test. Both treatments were held at 0.43-µg/L TBT.

	Dose (0.43-µg/L TBT)	
Hour	Leachate	Pure compound
0	100	100
24	100	100
48	92	100
72	72	84
96	48	56
120	36	36
144	8	20
168	4	16

LONG-TERM MORTALITY

The highest mortality across all dose levels occurred during the first 22 days of the test, or as the mysids were passing through the juvenile, and sub-adult stages (table 5, figure 4). All animals died in the highest dose level (0.48-µg/L TBT) within the first 7 days.

Table 5. Percent survival throughout 63-day long-term mortality test.

	Dose (µg/L TBT)					
Day	Control	0.03	0.08	0.25	0.38	0.48
0	100	100	100	100	100	100
22	86	82	70	90	75	0
29	84	82	70	90	67.5	0
36	76	82	68	90	65	0
41	72	80	68	86	55	0
49	68	76	66	86	52.5	0
53	62	74	64	80	47.5	0
63	60	72	60	74	22.5	0

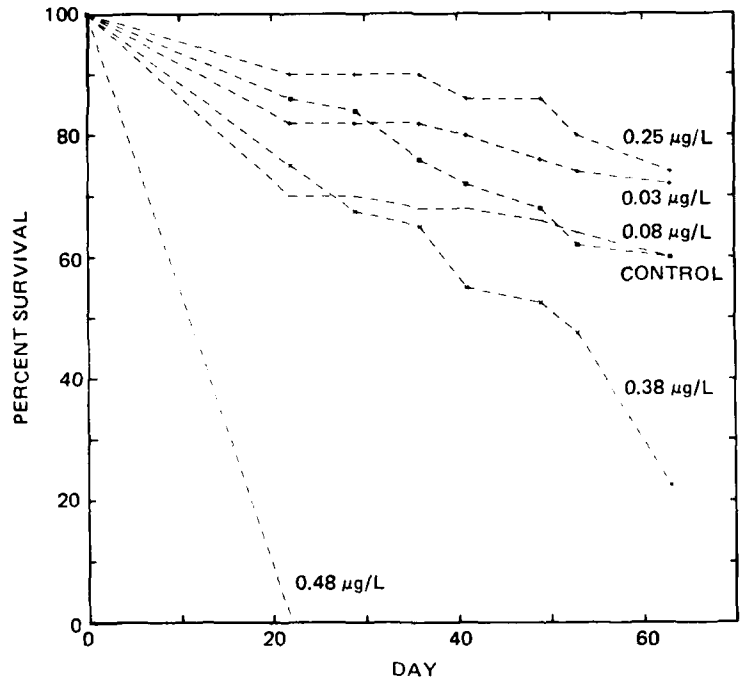

Figure 4. Long-term mysid mortality test – percent survival through time.

For each period observed, an analysis of variance was performed testing for differences in the number dead between concentrations (a=0.05).

Day 22 was the first day actual live counts were taken in each container. Counts previous to this day were of dead specimens only. This was done to minimize disturbing mysids due to their small size at this point in the test. No significant differences were noted in mysid survival within the four remaining concentrations and seawater control up to test day 22. A No Observable Effects Concentration (NOEC) was placed at 0.38-µg/L TBT, the highest concentration where no significant differences in survival were observed.

Day 41 was the last day observations were made prior to females releasing young. No significant differences in toxicity between dose levels were determined at this point, and the NOEC for 22 days (0.38-µg/L TBT) remained valid for the 41-day interval.

From day 41, survival decreased in the 0.38-µg/L TBT dose level at a faster rate than in the other levels. On the last day of the test (day 63), survival was recorded at 22.5 percent compared to 60 percent in the controls. A significant difference existed between the 0.38-µg/L dose and all other doses, which lowered the NOEC estimate to 0.25-µg/L TBT for an entire life cycle of *A. sculpta*.

GROWTH

Growth data presented here summarize a series of four tests where growth measurements were taken (tables 6 through 9). An analysis of variance was performed separately on weight and length within each set of data. A separate analysis was performed for each day measurements were taken. After the mysids reached sexual maturity, separate analyses were performed for each sex since females proved significantly larger than males of the same age group. The data were tested at an alpha level of 0.05 for differences between concentrations. Where a statistically significant difference was found, a Duncan's multiple range test was performed to specify those concentrations that were different.

Table 6. Final mean lengths and weights from long-term mortality test.

Day	Sex	Dose	TBT exposure (µg/L)	n length	Mean length (mm)	n weight	Mean weight (mg)
63	F	0		42	10.32	41	4.64
		1	0.03	11	10.27	11	4.71
		2	0.08	10	10.65	10	4.85
		3	0.25	19	9.98	18	4.27
		4	0.38	7	9.40+	7	3.40+
	M	0		50	9.42	50	3.32
		1	0.03	25	9.36	25	4.11
		2	0.08	20	9.25	20	2.85
		3	0.25	18	8.89	18	2.68
		4	0.38	3	8.73	3	2.52

+ Significant difference from other dose levels

Table 7. Mean length measurements from mysid growth test no. 1.

Day	Sex	Dose	TBT exposure (µg/L)	n	Mean length (mm)	Mean weight (mg)
0	-	-		30	2.03	*
6	-	0		10	2.69	
		1	0.03	10	2.80	
		2	0.08	10	2.83	
		3	0.20	10	2.95	
		4	0.38	10	2.83	
14	-	0		8	4.37	
		1	0.03	8	4.30	
		2	0.07	8	4.39	
		3	0.19	8	4.07\+	
		4	0.33	8	3.99/+	
27	-	0		8	7.50\+	
		1	0.33	6	7.71/+	
		2	0.07	8	6.73\+	
		3	0.20	8	6.77/+	
39	F	0		5	9.92	
		2	0.07	5	9.12\+	
		3	0.25	6	8.82/+	
	M	0		3	8.07	
		2	0.07	3	7.40	
		3	0.25	2	8.10	
48	F	0		1	11.00	
		2	0.07	17	10.13	
		3	0.24	6	10.40	
	M	0		2	9.10	
		2	0.07	9	8.33	
		3	0.24	5	8.80	

* Weight not measured during this test
\\+ Begin similar group significantly different from other doses
/+ End similar group significantly different from other doses

Table 8. Mean length and weight measurements from mysid growth test no. 2.

Day	Sex	Dose	TBT exposure (µg/L)	n length	Mean length (mm)	n weight	Mean weight (mg)
0	-	-		31	1.88		*
10	-	0		16	3.59	13	0.158
		1	0.04	6	3.83	6	0.166
		2	0.10	9	3.72	8	0.167
		3	0.18	6	3.49	2	0.120
		4	0.32	9	3.44	7	0.164
		5	0.60	5	2.96+	4	0.094
28	F	0		5	8.36	5	2.10
		1	0.03	6	7.29	6	1.88
		2	0.08	3	7.40	3	1.67
		3	0.15	4	7.61	4	1.70
		4	0.27	5	7.50	5	2.04
		5	0.49	2	4.69+	2	0.45+
28	M	0		3	7.36	3	1.39
		1	0.03	4	6.33	4	1.00
		2	0.08	5	6.54	5	1.09
		3	0.15	7	7.37	7	1.40
		4	0.27	3	6.74	3	1.39
		5	0.49	1	5.73	1	0.71

* Mysids too small to weigh
+ Significant difference from other doses

Table 9. Mean length and weight measurements from mysid growth test no. 3.

Day	Sex	Dose	TBT exposure (µg/L)	n	Mean length (mm)	Mean weight (mg)
0	-	-		11	1.42	0.026
1	-	-		12	2.07	0.047
8	-	0		7	2.25	0.051
		1	0.04	6	2.05	0.090
		2	0.15	8	2.42	0.061
		3	0.24	10	2.87	0.117
		4	0.24	13	2.69	0.105
21	-	0		6	4.83	0.407
		1	0.05	2	4.85	0.423
		2	0.14	6	5.31	0.541
		3	0.20	10	4.99	0.505
		4	0.31	6	5.07	0.571

Sexually mature females in the higher dose levels tended to exhibit less growth than their counterparts in the lower dose levels and sexually mature males in all dose levels. This is most apparent from the measurements made on the survivors of the long-term mortality test. Both length and weight were significantly lower in the 0.38-µg/L TBT level than any of the lower levels. In the second growth test, females showed a significant difference in both length and weight at the 0.49-µg/L TBT level. No such differences were seen in the corresponding males.

The first growth test indicated that 39-day-old females exhibited a statistically significant growth inhibition in length at TBT concentrations ranging from 0.25 to 0.07 µg/L. The significance of this result was somewhat questionable, however. These smaller lengths were not consistent with results from other similar tests. Final measurements from the long-term mortality test showed growth inhibition in adult females at 0.38-µg/L TBT. Growth test 2 showed growth inhibition in 28-day-old females at 0.49-µg/L TBT. Additionally, the results reported in the first growth test were based only on length measurements when the aforementioned, comparable tests were based on both length and weight measurements. Length measurements tended to be more variable than weight measurements and were not as good an indicator of growth effects. Length and weight measurements combined, however, gave a better indication of growth than either did separately. Finally, the statistical significance determined was based on a small sample size and hence does not warrant a great amount of confidence. Therefore, because these length measurements were taken from a small sample size and were not consistent with results of similar tests, this result was not included in the calculation of final chronic values.

In addition to the obvious differences in growth of adult female mysids, an apparent trend exists that suggests TBT at the higher dose levels tested (0.4- and 0.5-µg/L TBT) inhibited growth and development of juvenile and subadult *A. sculpta*. A significant difference was detected in length at day 14 in the first growth test where mean lengths of 4.07 and 3.99 mm in 0.19- and 0.33-µg/L TBT levels, respectively, were significantly less than the mean length (4.37 mm) measured in the controls (table 7). Data from day 27 of the same test also showed a significant lower mean length of 6.77 mm at 0.20-µg/L TBT compared to 7.50 mm in the controls. During the second growth test, a significant difference was found in length at the 0.60-µg/L TBT level between the average test specimen length (2.96 mm) and the controls (3.59 mm) (table 8). The third growth test was conducted at 0.31-µg/L TBT, the highest concentration tested, and showed no growth effects on subadults (table 9).

Therefore, considering the responses obtained, two NOEC values can be estimated for growth—one for subadults and one for adults. The NOEC estimate for subadults is based on the response of no inhibited growth at 0.31-µg/L TBT by day 21 in growth test 3. The NOEC for adults is based on the response of females showing inhibited growth at 0.38-µg/L TBT after 63 days during the long-term mortality test. This lower NOEC determined for adult females (0.25 µg/L) from the long-term mortality test compared to the NOEC calculated for subadults (0.31 µg/L) from growth test 3 was probably influenced by the increased exposure time to TBT during the sensitive period of maturation and reproduction.

REPRODUCTION

In the reproductive success test, individual, gravid females were held in separate replicate containers within a TBT dose level, and four parameters of reproduction were observed. The number of juveniles released per female and the number of individuals in unhatched broods were counted, the number of days from the hatching of a female to the release of its juveniles was noted, and the number of females that actually released juveniles was observed.

A separate analysis of variance was performed testing the number of juveniles released per female and the number of individuals in unhatched broods between concentrations (a=0.05). In addition, the number of juveniles released by day was tested between concentrations as well as the number of days from the hatching of a female to the release of its juveniles. The success of each female's hatch was measured by a yes/no response and was tested using a Chi-square test for a two-way contingency table (hatch X dose). An analysis of variance was also performed on the total number of juveniles produced in each test concentration from the 14 test mysids.

All test organisms in the highest dose level (0.52-µg/L TBT) died as juveniles and subadults within the first 12 days of the test. There were no significant differences in the number of juveniles released per individual female, the number of individuals in unhatched broods (from those individuals holding broods), or the number of days from hatching of a female to the release of its juveniles in the remaining four dose levels and seawater control. Of the females that released juveniles in all dose levels, the average number released was 11, ranging from 10 at the 0.09-µg/L level to 12 at the 0.03-µg/L level (figure 5, table 10). Of the remaining females through-

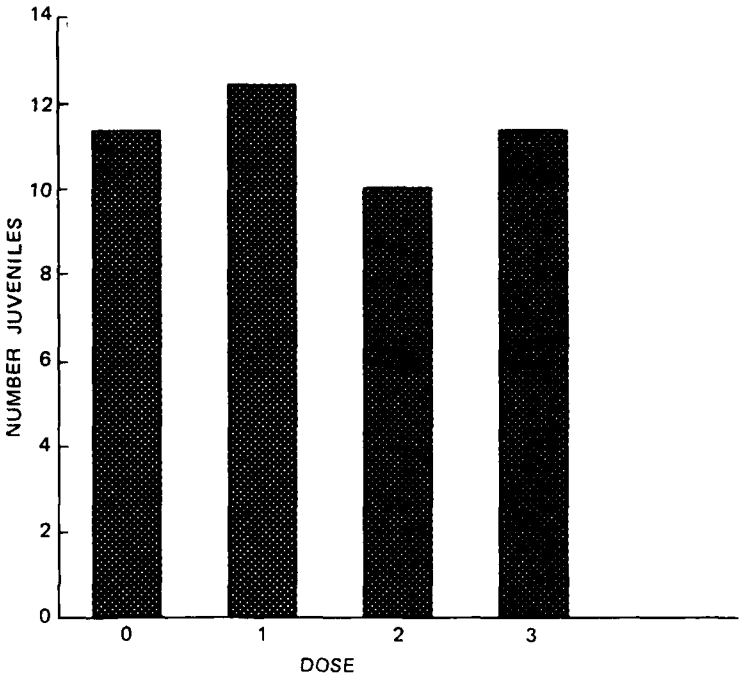

Figure 5. Reproductive success bioassay test – average number of juveniles hatched per female (only individuals releasing juveniles included).

Table 10. Number of viable juveniles released from select females.

Female ID number	Dose (µg/L TBT) Control	0.03	0.09	0.19	0.33	0.52
1	10	17	13	0	0	-
2	10	0	6	0	0	-
3	0	11	10	0	0	-
4	17	0	14	13	0	-
5	0	2	3	0	0	-
6	5	0	7	0	0	-
7	0	6	10	0	0	-
8	0	17	20	14	0	-
9	0	1	5	7	0	-
10	0	0	10	0	0	-
11	18	10	0	0	0	-
12	0	21	12	0	0	-
13	8	19	0	0	0	-
14	*	20	*	*	0	-

* Data not collected
- Individual dead before sexual maturity

out the entire test holding broods, the average number of juveniles in each brood was 14, ranging from 3 at the 0.33-µg/L dose level to 16 in the controls. The average number of days from the hatching out of a female to release of its young was 43 days, ranging from 41 days at the 0.33-µg/L dose level to 44 days at the 0.19-µg/L dose level (table 11). No significant differences were determined in the above mentioned parameters.

Table 11. Number of days from hatch of female to release of juveniles.

Female ID number	Dose (µg/L TBT) Control	0.03	0.09	0.19	0.33	0.52
1	46	46	43	.	.	-
2	47	.	47	.	.	-
3	.	42	45	.	.	-
4	42	.	42	42	.	-
5	.	42	41	.	.	-
6	41	44	46	.	.	-
7	.	46	53	.	.	-
8	.	41	41	40	.	-
9	.	43	41	40	.	-
10	.	.	44	.	.	-
11	40	40	.	.	.	-
12	.	41	42	.	.	-
13	40	42	.	.	.	-
14	*	45	*	*	.	-

* Data not collected
. Individual did not release juveniles
- Individual dead before sexual maturity

However, there was a significant and obvious difference in the number of test females that actually released viable juveniles. Of the 14 test females held in each dose level, none released juveniles at the 0.33-µg/L level and only three released at the 0.19-µg/L level (figure 6). Although the 0.09- and 0.03-µg/L levels showed a higher number of females releasing compared to the controls, the 0.33- and 0.19-dose levels showed a significant difference compared to the other doses combined.

Posttesting examination of the individuals that did not release juveniles revealed that many of the brood pouches were empty and those that were not contained dead eggs. Females holding no eggs may have produced eggs and aborted them shortly afterwards, since most females had been observed holding eggs at some time.

A significant difference in the number of juveniles produced was noted at the 0.33- and 0.19-µg/L dose levels. A NOEC for effects on reproduction was estimated at 0.09-µg/L TBT.

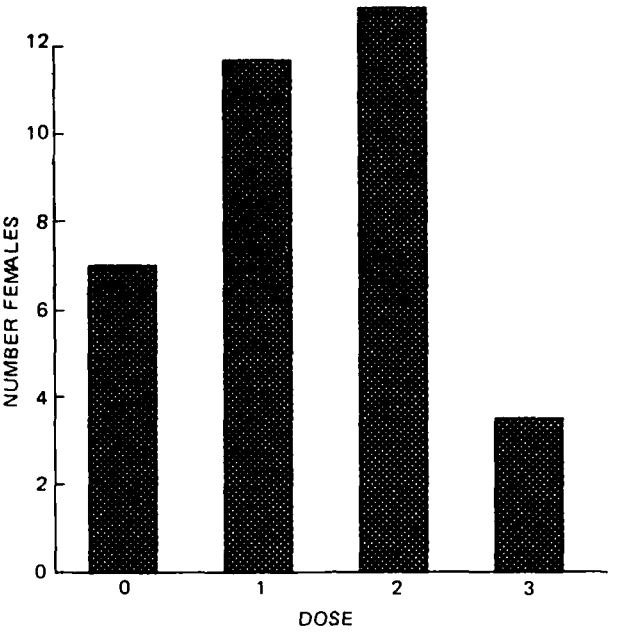

Figure 6. Reproductive success bioassay test – number of females releasing viable juveniles.

DISCUSSION

STATISTICAL CONSIDERATIONS

Statistical tests used in chronic toxicity studies are selected on the basis of their application to the response under consideration and their ability to detect differences between the responses of the control and the treatment groups. Biologists have traditionally been criticized by statisticians for using, or misusing, certain statistical procedures for testing biological responses (e.g., Dawkins, 1983; Jones, 1984; Preece, 1982; and Finney, 1982). The problem arises when trying to apply the mathematical science of statistics with its rigorous assumptions for testing to complex biological responses and the economic and physical restraints often encountered in trying to measure these responses.

A single toxicity experiment designed for and analyzed by a particular statistical test may not be appropriate when the experiment is completed, depending upon the biological response observed. Nor does a single test measuring a single response necessarily accomplish a great deal in achieving the overall goal of aquatic toxicology testing: "the protection of diverse aquatic organisms and whole ecological communities from the dire effects of

man-made chemicals" (Dagani, 1980). Aquatic toxicology testing is costly, and, depending upon the organism(s) being tested, requirements for holding and rearing test animals may be impractical. Therefore, most testing is designed to measure several response parameters, and innovative means are continually being employed to analyze these responses singularly and collectively to estimate cumulative effects on ecology (Javits, 1982; Capizzi et al., 1985).

The goal or outcome of any chronic toxicity test is the estimation of a NOEC or NOEL. This concept was first introduced by Mount and Stephan (1967) in an attempt to standardize the reporting of toxicity levels of pollutants. They developed the term Maximum Acceptable Toxicant Concentration (MATC) and defined it as that concentration level in which the fish production rate is not greatly different from that present in an uncontaminated environment. Citing ambiguity in the acronym MATC, Maki (1979) proposed the term NOEC "as a logical replacement term for MATC implying no change in the manner of determining the concentration but simply a clarification of its interpretive significance." Payne and Hall (1979) use NOEL in place of NOEC. All terms are used in the literature, and the terms report the highest concentration of a toxicant tested that showed no significant difference in response as compared to controls.

Investigators determine a NOEC for a particular toxicant on a particular organism using various methods depending on the parameters observed and the responses obtained. Javits (1982) suggests that the determination of a NOEC requires more than the application of statistical tests: both the nature and magnitude of the adverse effects should be taken into account. He suggests the application of biological judgment in conjunction with statistical procedures be used to determine a NOEC.

The statistics applied to the responses observed in these mysid toxicity tests were selected for their straightforward approach, applicability to the responses observed, and past use in toxicology testing and similar studies. Biological judgment is used in interpreting the results.

Probit Versus NOEC

Mortality in the short term is considered an acute effect of a toxicant and is measured by means of a test rendering a 96-hour LC_{50} by means of a probit analysis. In long-term, chronic tests, sublethal effects can be accentuated through time and can contribute to the death of an animal. A probit analysis can be applied to any test that provides dose-dependent mortality data for a particular time. Application of a probit analysis on the long-term mortality test performed here was not appropriate because the mortality response throughout the test was not entirely dose-dependent.

A direct, dose-dependent response was not observed in this test at concentrations of 0.25-µg/L TBT and below. Within concentrations of 0.03-, 0.08-, and 0.25-µg/L TBT, survival was greater than in the control. Such a response can be attributed to hormesis, a tendency for some toxicants to stimulate survival, growth, and reproduction at subacute concentrations. The phenomena of hormesis is not well understood, but it has been widely observed in chronic toxicity testing (Stebbing, 1982), and an effect of this nature has been observed in all chronic mysid tests performed here.

Because of the observed hormesis effect, probit analysis could not be performed with the long-term mortality test data and trend tests performed by Capizzi (1985) could not be applied to growth or reproduction parameters. Therefore, the more traditional analysis of variance, which compares dose levels individually together with multiple range tests which group similar dose levels, was used in the majority of the chronic testing performed to determine significant differences at the a=0.05 level. These procedures were described by Duncan, 1965; Williams, 1971, 1972; and Dawkins, 1983.

Replicates Versus Duplicates

The flowthrough dosing system used in all chronic toxicity tests performed was described in detail by Valkirs et al. (1985a). In terms of statistical analysis, the replicates within each dose level in this system are not true replicates but rather duplicates. This is a result of each replicate container being fed by a common dose source, the leaching trough via the distribution trough. True replicates would be dosed independent of each other and would be able to be distributed randomly throughout a laboratory to minimize any error due to spacial distribution. In this system, replicates are confined to corresponding dosing racks even though random distribution is possible within these racks.

The use of true replicates permits determination of experimental error imposed by the dosing system and physical laboratory configuration to subtract that error in statistical analysis resulting in a better view of the actual response due to the toxicant (Hurlbert, 1984; Rowell & Walters, 1976). We have found by performing many TBT toxicity tests in this laboratory that the replicate, or duplicate, variability observed in these tests is minimal compared to the toxicant responses obtained. In chronic tests where dose levels are low and relatively similar, the biological responses between doses at the NOEC level are usually dramatic and of a magnitude far greater than any variability observed within dose levels.

The replicates used here mimic more, in practice, true replicates than the duplicates suggested by Javits (1982), subdivided aquaria, for increasing the power of a statistical test. Although our test containers receive dose water from a common source, there is no direct pathway between test containers for this water to flow once it has entered a container, thereby eliminating cross-contamination of containers. Additionally, physical parameters within the testing lab such as temperature and light are controlled and constant throughout the small space of the lab. There is little evidence to suggest that random distribution within a dosing rack would not accomplish the same results as random distribution throughout the lab.

Therefore, in the statistical analyses performed, our replicates, or psuedo-replicates, have been treated as true replicates to take advantage of the increased statistical power offered. The result is an example applying practical biology to the rigors of statistics while using sound biological judgment.

ACUTE TOXICITY

A 96-hour LC_{50} has previously been estimated for the mysid *A. sculpta* at 0.61-µg/L TBT (Valkirs et al., 1985a) using a flowthrough dosing system. The current static renewal acute toxicity test was performed to establish a potentially better LC_{50} estimate by minimizing dose fluctuations and better estimating actual TBT concentrations by sampling more frequently.

We have observed in previous tests with *A. sculpta* that acute TBT doses took 1 to 5 days to exhibit a mortality response. During that time, individuals exhibited a stressed condition through slow movement, aberrant swimming behavior, and, in many cases, no movement unless prodded. Such a response was observed in this test at dose levels of 0.43-, 0.54-, and 0.66-µg/L TBT.

The ability of this species to recover from exposure to concentrations below the LC_{50} determined by static renewal (0.42-µg/L TBT) when placed in clean seawater, but not from exposure to concentrations above 0.42 µg/L illustrates that the 96-hour LC_{50} for *A. sculpta* is more than just an arbitrary, standardized acute toxicity value. It represents an actual value below which these mysids can recover after a 96-hour exposure period and above which recovery may not occur.

As illustrated by the acute test and the associated spike test, exposure time to TBT directly affects the ability of a mysid to recover. After 96 hours, mysids exposed to concentrations above 0.42-µg/L TBT did not recover, while after an 8-hour exposure to 1.13-µg/L TBT surviving mysids could recover when returned to a subacute dose. The immediate mortality response observed during the 1.13-µg/L spike suggests the mechanism for toxicity at this higher acute level may be different and more direct than the mechanism involved in the lower acute levels.

The possibility of synergistic effects involving TBT and other known toxicants found in the leachate used for dosing was considered, particularly since copper is also contained in the antifouling paint tested. The low toxicity of copper relative to TBT has been discussed by Uren (1983). Copper concentration (2 µg/L) measured in the highest leachate concentration used in our studies was well below reported toxic levels. But the synergistic, or combined, effects of both copper and TBT may amplify the toxicity of each. To investigate this possibility, the leachate/pure compound test was performed in conjunction with the acute toxicity test comparing the toxicity of the TBT aleachate solution used in all tests and a solution containing only TBT established from a pure compound.

The mortality curves obtained from this test proved similar throughout the 96-hour dosing and 72-hour recovery phases with both leachate and pure compound solutions. This suggests that other toxicants leaching from the painted panels, including copper, do so at sublethal levels that do not act synergistically with TBT.

LONG-TERM MORTALITY

The long-term mortality test was performed with *A. sculpta* to investigate the effects of TBT on mortality throughout a life cycle. Mortality in the short term is considered an acute effect of a toxicant and is measured as in the acute toxicity test described previously. However, sublethal effects can become accentuated through time and contribute to the death of a species. Also, specimens may be more sensitive to a toxicant during various stages or periods during their life cycle that may be excluded during a short-term test. Juvenile *A. sculpta* are nearly three times more sensitive to TBT than are adults (Valkirs et al., 1985a). However, adults may become more sensitive during periods of molting, mating, or reproduction, which a life cycle mortality test may illustrate.

An LC_{50} value for various periods throughout this test could not be estimated because the mortality data did not prove to be dose dependent. Survival was higher in dose levels up to and including 0.25-µg/L TBT than in the seawater control. This is an apparent effect of hormesis, a tendency for some toxicants to stimulate growth and reproduction at subacute concentrations. The phenomena of hormesis is not well understood, but has been widely observed in chronic toxicity testing (Stebbing, 1981, 1982; Laughlin, 1981; Newton et al., 1985). An effect of this nature was observed in all chronic mysid tests performed.

A decrease in the NOEC after females began releasing juveniles suggests these mysids may become more sensitive to TBT during the release of young. Even though the sex of dead mysids was not determined (dead individuals were usually cannibalized beyond recognition during observations), we have observed that a dead female was usually present in a container containing new juveniles. Therefore, the increase in mortality noted was assumed to be due to female mortality after release of juveniles.

GROWTH

The effect of a toxicant on the growth of an organism is a chronic effect that can affect an organism's ability to compete for food, mate, reproduce, and avoid predation. Decreased growth of individuals can affect the total biomass produced by a population and, hence, affect the food chain community (Clutter & Theilacker, 1971; Hobson & Chess, 1976). Growth is a parameter typically investigated in chronic toxicity testing.

Throughout these tests, a hormetic effect was apparent that caused growth values in several dose levels to exceed those recorded in the controls. These results tended to cloud the analyses, producing some interesting multiple-range groupings. However, some obvious significant differences were noted.

Results from dose levels that approached and exceeded the 96-hour LC_{50} determined and exceeded the NOEC for long-term mortality showed that mysids exhibiting decreased growth rate also had a high mortality rate. The effects of growth manifested in this study were likely a result of a general stress condition rather than the result of any direct mechanism of growth inhibition. Females appeared more susceptible to TBT-induced stress than males.

REPRODUCTION

The effect of a toxicant on the reproductive ability of an individual is a chronic effect on the population. Although a particular concentration may not impair an individual's ability to survive and grow, if it affects its ability to reproduce, it affects the integrity of the population. Reproductive success is typically included in chronic toxicity studies and is measured in a variety of forms, depending on the organism tested and its response to the toxicant.

Data on reproduction of Mysidacea are easier to obtain than for most pelagic invertebrates (Clutter & Theilacker, 1971). Since the eggs and larvae are carried in a brood pouch, they are easy to enumerate and are a good measurement of reproductive ability. However, investigators have noted in toxicity studies with other species of Mysidacea that not only the number of young produced per female, but also delay in the formation of brood pouches and release of young were useful reproductive effects (Nimmo et al., 1977).

On an individual basis, the data on the number of juveniles hatched per female do not appear significant (table 10). However, if a significant number of females cannot release viable juveniles at the TBT levels tested, the overall recruitment of the population may be reduced and the population may suffer an effect. Figure 7 illustrates recruitment within the small test population by considering all females tested and all juveniles produced. While the number of juveniles released per hatching female was not significant between concentrations, an obvious reduction in overall reproductive success was noted at 0.19-µg/L TBT. This represents a potentially toxic concentration with respect to population recruitment.

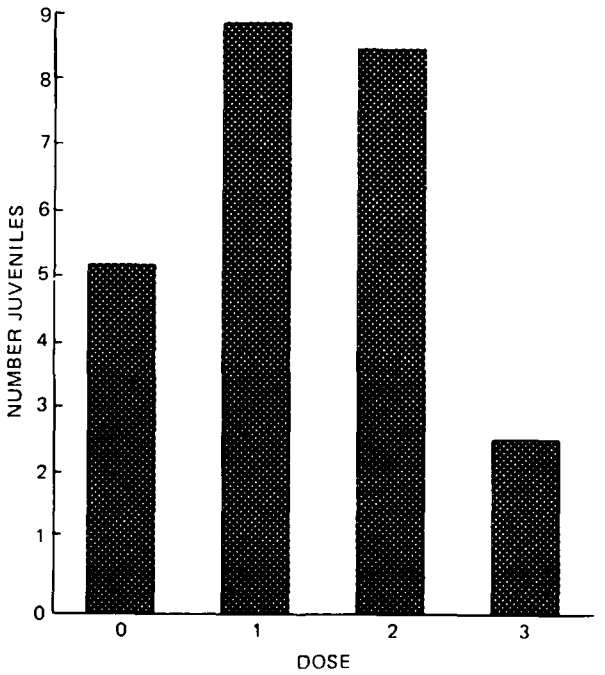

Figure 7. Reproductive success bioassay test – average number of juveniles hatched per female (all hatching and nonhatching females included).

CONCLUSIONS

FINAL CHRONIC VALUE

Various authors have used a variety of terms and definitions to interpret and report results of chronic tests, and care must be taken when chronic studies are reviewed. As mentioned previously, the NOEC has been used as a standard chronic value for parameters tested but has been referred to using several terms, including MATC (Mount & Stephan, 1967) and NOEL (Payne, 1979), without altering its basic definition. Stephan et al. (1985) modified the definition of a chronic value in calculating acute/chronic ratios as the geometric mean of the lower and upper chronic limits of a chronic test. The lower limit referenced here is the highest concentration of a toxicant tested that did not show an adverse effect (the actual definition of a NOEC). The upper limit is the lowest concentration tested that did show an adverse effect. This later method of determining a chronic value would then, of course, increase the concentration of a final value.

To clarify the chronic results of this study, chronic values are summarized in table 12. They include the lower chronic limits, or NOECs, the upper chronic limits, and the mean of the two for effects on long-term mortality, growth, and reproduction. From this summary it is evident that TBT affects the sexually mature females' ability to reproduce at a concentration of approximately 0.19-µg/L TBT, and their ability to grow is affected at a concentration of 0.38-µg/L TBT. The final NOEC, based on the most sensitive chronic indicator, is 0.09-µg/L TBT, and the best estimate of a final chronic value is 0.14-µg/L TBT.

Table 12. Summary of chronic values in µg/L TBT.

Test	Lower limit (NOEC)	Upper limit	Final chronic value
Long-term mortality	0.25	0.38	0.31
Growth - subadults	0.31	0.60	0.45
- adults (females)	0.25	0.38	0.31
Reproduction	0.09	0.19	0.14

ACUTE/CHRONIC RATIO

In calculating an acute/chronic ratio for mysids, Stephan et al. (1985) requires a final chronic value as described previously. He suggests the final acute value should be a 96-hour EC_{50}, the concentration of a toxicant at which 50 percent of the test animals are affected in some adverse way in 96 hours, based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized and the percentage of organisms killed. In place of an EC_{50}, if not available, they require a 96-hour LC_{50} be used in its place. The LC_{50} only measures mortality and not lesser effects.

The 96-hour LC_{50} estimate obtained from the static renewal acute toxicity test performed was 0.42-µg/L TBT. This value divided by the final chronic value obtained (0.14-µg/L TBT) gives an acute/chronic ratio of 3.0 for the mysid *A. sculpta*.

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